THE COMBINED EFFECTS OF DOSAGE LEVEL AND CS - UCS INTERVAL ON THE FORMATION OF ONE - TRIAL POISON-BASED AVERSIONS

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Abstract

The main concern of the present research was to study the combined effects of UCS strength and CS - UCS delay on poison-based aversions using intubated saline as the UCS. One hundred and sixty male albino rats were used in a 4 X 5 (Concentration x Delay) factorial design experiment. Saline concentrations were 0.9%, 2.7%, 8.1% and 12.15% (w/v), while CS - UCS delays were 0.5, 1.5, 4.5, 13.5 and 24.0 hr. Each animal was intubated with one saline concentration at one delay following presentation of 0.5% (w/v) sodium saccharin. All Ss were 24 hr. water-deprived throughout the experiment except during the three recovery days following intubation.

The results showed that all three experimental saline concentrations were effective (compared to isotonic saline control groups) in producing aversions at the three shortest CS - UCS delays on the first post-training preference test day. These aversions were of varying strengths and they extinguished at different rates. For example, aversions with the 12.15% solution were the strongest, those with the 8.1% solution the next strongest and those with the 2.7% solution the weakest. Extinction, measured in terms of the amount consumed on five successive test days,

followed a similar pattern with the 12.15% groups extinguishing slowest and the 2.7% groups fastest. Gradients of aversions related to the CS - UCS interval were not quite so orderly in that the 0.5, 1.5 and 4.5 hr. groups all showed approximately the same level of aversion on the first test day. Moreover, no delay longer than 4.5 hr. led to a significant aversion at any of the saline concentrations used.

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Introduction

Paradigm used in poison-based avoidance learning

The paradigm which is used to demonstrate poison-based avoidance learning is a very simple one. Ingestion of a harmless food (CS) is followed, after some period of time, by sickness (UCS). Upon complete recovery from the sickness, Ss are allowed to consume the same food substance and, when comparisons are made with appropriate controls, it is typically found that they now avoid the food which preceded the induced sickness.

The use of induced sickness as a UCS in learning studies developed during the 1950's from observations which showed that radiation sickness could be produced in animals and used as an unconditioned stimulus (Garcia, Kimeldorf, Hunt & Davies, 1956; Garcia, Kimeldorf & Koelling, 1955; Leary, 1955). Even earlier than this sickness caused by lack of an essential substance, such as thiamine or another vitamin, had been observed to lead to a change in an animal's choice of diet (e.g., Harris, Clay, Hargreaves & Ward, 1933; Richter, 1936; Richter, Holt & Barelare, 1937; Young & Chaplin, 1945). The cause for this dislike of a deficient diet was thought at first to be instinct, and more emphasis was placed on the increased preference for any novel diet given the animals at this time than on the dislike for the deficient one. More recent work, however,

has shown that the change in preference is based on more general learning principles (e.g., Rodgers & Rozin, 1966; Rozin, 1967; 1968; 1969a; Rozin & Rodgers, 1967; Zahorik & Maier, 1969) insofar as the change is produced by slow poisoning from the deficient diet. Hence it has been suggested that preference changes caused by deficient diets and those caused by the more rapid sickness induced by radiation may be two forms of poison-based avoidance learning (e.g., Rozin, 1968; Rozin & Kalat, 1971).

The two main differences between poison-based avoidance learning and traditional forms of learning appear to be the number of training trials and the time between CS and UCS that will produce a learned response. Fewer training trials, often only one, are typically successful in producing poison-based aversions. This in itself is not unusual since one trial learning can and does occur in passive avoidance situations (e.g., Essman & Alpern, 1964). In avoidance conditioning, however, one trial learning is the exception rather than the rule. Moreover, in poison-based avoidance learning, one trial aversions are learned over CS - UCS intervals of several hours. These two differences, in the past, have been the main reasons for much of the skepticism with which many have viewed poison-based avoidance learning. In fact, most theories of learning assume that the main

pre-requisites for learning are the occurrence of many reinforced training trials and close temporal contiguity of the stimuli to be associated. As a result, the first reaction of some was to attribute the unusual findings of poison-based avoidance learning to such artifacts as habituation and sensitization, or to mediation by after-tastes or secondary reinforcers.

Habituation would explain the results of poison-based avoidance learning by positing that the animal did not form an aversion to the substance but simply became tired of consuming it because of repeated presentations. However, many poison-based avoidance learning studies have included groups of subjects which received the same exposure to the test substance, but underwent no induced sickness (e.g. Farley, McLaurin, Scarborough & Rawlings, 1964; Garcia, Ervin & Koelling, 1966; Garcia, Kimeldorf & Hunt, 1961; Kral, 1970; McLaurin, 1964; McLaurin & Scarborough, 1963; Revusky, 1968; Smith & Birkle, 1966; Smith & Roll, 1967). In these experiments repeated exposure to the test substance alone was not enough for an aversion to it to occur. In fact, there is evidence in some of these studies that preference for the test substance actually increased if the substance was not followed by aversive stimuli.

Sensitization is not the explanation of the results of poison-based avoidance learning studies either. That is to say, it could be argued that presentation of the punishing or aversive stimulus without prior occurrence of the response could be enough to lead to reduced consumption of the test substance. There are many studies, though, which show that if the test food is not consumed prior to the induced sickness no aversion to the test food is formed (e.g., Garcia, Ervin & Koelling, 1966; Garcia, Kimeldorf & Hunt, 1961; Revusky, 1968; Smith & Birkle, 1966; Smith, Taylor, Morris & Hendricks, 1965). The only possible exception to this finding occurs if the CS is presented after the UCS, but while the subject is still suffering the effects of the induced sickness (e.g., McLaurin, 1964). Here animals that underwent such a treatment did form an aversion. McLaurin argued that this was evidence that poison-based aversions were not a normal form of learning, and that, in fact, sensitization could explain the findings. This, however, was not the case because subjects had experienced the shortest CS - UCS delay possible. That is to say, the preference tests started immediately after radiation exposure so that subjects consumed the test solution (CS) when they were still suffering the effects of the radiation sickness, which is known to last for some hours after exposure (Revusky & Garcia, 1970). The sickness was, therefore, contiguous

with the CS. Hence, even in this instance, there is no evidence to support the use of sensitization as an explanation of poison-based avoidance learning.

The other two possibilities which have been suggested as alternatives to poison-based avoidance learning are that the response is mediated by aftertastes or by secondary reinforcers. It is known from more traditional learning situations that if learning is to occur with long delays between response and reinforcement some cue must be present to help the animal mediate the temporal gap. For example, both Shilling (1951) and Harker (1956) found that lever pressing could be maintained with delays of reinforcement up to five and ten seconds, respectively. This ability was, however, correlated with the rat maintaining its orientation toward the lever during the delay interval which, in some way, bridged the time between the response and reinforcement for the animal. The most obvious way that flavor aversions could be mediated is by aftertastes. That is to say, the flavor of the test substance is still experienced by the rat at the time the sickness is induced, hence eliminating the effective CS - UCS interval. The likelihood of this happening becomes very remote when one considers some of the delays involved. In the experiments by Nachman (1970a), Revusky (1968) and Smith and Roll (1967), for example, the CS - UCS

intervals of some of the groups tested were at least six hours. After an interval of this length, the substance, such as sucrose, has been digested by the stomach and the only trace of it would be the slight rise in blood sugar level which it would be impossible for the rats to taste. This fact alone should be enough to render the aftertaste hypothesis unsatisfactory. It was also argued that an aftertaste could be provided by the induced sickness leading to the animal vomiting the CS, again causing close temporal contiguity of CS and UCS. However, regurgitation of the CS is not a tenable explanation either since, as Garcia and Ervin (1968) observe, rats can not vomit. Even if rats could regurgitate, the CS has already been digested with some of the CS - UCS delays at which aversions have been found. Thirdly, aversions have been found in studies where the only CS present was a small amount of a hydrochloric acid solution which could not have produced a noticeable change in the hydrochloric acid level of the stomach and hence could not have been detected by aftertastes (see Revusky & Garcia, 1970). Finally, Nachman (1970a) has obtained aversions using temperature of water as the CS, thus precluding the possibility of aftertastes occurring at all. The conclusion from these studies, and others, can only be that poison-based aversions can not be explained with mediation by aftertastes as an explanation.

The other possible source of mediation is by secondary punishment. That is to say, the immediate physiological results of eating become secondary punishers by being paired with primary punishment. In this way, secondary punishers can mediate the time delay between CS (eating) and primary punishment (sickness). 'In order for this to occur, however, the primary punishment and the CS would have to be paired on more than one training trial, since the ingestion of the CS can not serve as a secondary punisher to mediate learning over long delays unless it has previously been paired with sickness. Hence, such an explanation is negated by the growing number of studies that have obtained aversions in one training trial over long delays (e.g., Nachman, 1970a; Rewusky, 1968; Smith & Roll, 1967).

The evidence cited against the occurrence of habituation, sensitization, mediation by aftertastes and mediation by secondary punishers by no means exhausts the number of studies that have been done in the area of poison-based avoidance learning. For this reason Table 7 (Appendix) has been included to give the reader an indication of the number of experiments that have produced flavor aversions with various toxins. Many of these studies also include the control groups necessary to rule out the presence of artifacts in poison-based avoidance learning.

Having clearly established that poison-based aversions are a learned phenomenon, researchers have shifted their interest in more recent years. The main concern of recent work has become the comparison of the similarities and differences between the poison-based avoidance paradigm and more traditional forms of learning such as classical and instrumental learning. As already noted the main differences between the learning of poison-based aversions and the learning of more conventional classical and/or instrumental responses appear to be the occurrence of one trial and long delay learning within the framework of the poison-based avoidance learning paradigm. However, it is becoming clear that many other phenomena that are found in more traditional paradigms also occur in a similar way in the poison-based avoidance situation. For example, Revusky and Garcia (1970) have reviewed the literature and concluded that, when floor and ceiling effects are not allowed to obscure the data, the usual relationships are found between intensity of UCS's, strength of aversion formed, and number of trials necessary to produce an aversion. Moreover, typical delay of reinforcement gradients are found when the CS - UCS interval is extended sufficiently (e.g., Smith & Roll, 1967). Finally, Revusky (1971) has reviewed evidence which shows that phenomena such as overshadowing, blocking, latent inhibition and sensory preconditioning occur in the poison-based avoidance

learning paradigm as well as in classical and instrumental learning paradigms. These learning phenomena, like the ones above, seem to behave in the same way in the various paradigms when the parameters are adjusted appropriately. For instance, overshadowing can be shown to occur as follows. Rats who had been given saccharin and were made sick one hour later were given various concentrations of vinegar to drink during the CS - UCS interval. The stronger the vinegar concentration the weaker the aversion that was formed to saccharin. The vinegar taste had overshadowed the taste of the saccharin and interfered with the formation of the aversion (Revusky, 1971).

As well as establishing that poison-based aversions are in fact learned aversions, and demonstrating the similarities between poison-based avoidance learning and other forms of learning, researchers in the area of poison-based avoidance learning have tried to explain the reason why one trial and long delay learning are possible and are often the rule rather than the exception in poison-based aversion learning. The principles postulated to explain these phenomena include stimulus relevance (Capretta, 1961), belongingness (Garcia & Koelling, 1966), preparedness (Seligman, 1970), and the failure of concurrent interference (Revusky, 1971). In general, each of the explanations uses some aspect of the relationship of the CS (taste) and the UCS (sickness) to

each other and of their relationship to the animal's ability to perceive them. This contrasts with older ideas that the use of conditioned stimuli totally unfamiliar to the subject would lead to a less contaminated learning situation and that, in theory, all stimuli have the same likelihood of being associated with each other. The newer arguments tend to center in one way or another around the idea that the taste - sickness association is more "relevant" (i.e., more natural) for the animal than many of the other CS - UCS associations that animals have been required to make in the past. It should also be noted that these explanations are not entirely different from each other. For example, what Capretta (1961) has called stimulus relevance is very similar to what Garcia and Koelling (1966) have called belongingness and to what Seligman (1970) has called preparedness. The next section will, among other things, deal in detail with the principle of stimulus relevance and the particular experimental paradigm used in its development.

The use of intubated saline as a toxin in the development of stimulus relevance as an explanatory principle in poison-based avoidance learning

The principle of stimulus relevance, as stated by Capretta (1961), was the first attempt to account for the apparent ease with which associations between flavor and

induced sickness were made. It stated that "certain associations are formed more easily if the events to be associated are capable of being perceived as belonging together (Capretta, 1961, p. 241)." It was adopted to account for the results of a study in which sickness caused by the intubation of saline was an effective stimulus in the conditioned modification of food preferences with chickens whereas shock to the feet of rats was not effective in producing food preference modification. In the study, chicks had experienced colored food associated with stomach loadings of milk, salt water or plain water prior to being fed one of two colored mashes. The association of the salt load with a particular color of mash led to a reduced preference for food of that colour whereas the milk and water loads did not lead to changed preference values for the food. The rats, on the other hand, were part of a pilot study carried out by Capretta (1961) and were given electrical foot-shock whenever they consumed one of two sugar - saccharin solutions. There were no marked changes in the rats' preference for the solutions. When, however, rats were intubated with noxious saline before consumption of the preferred solution there was a significant reduction in the preference value of the solution.

A subsequent study by Braveman and Capretta (1965) provided further support for the relevance principle. Here rats were

used in an experiment in which salt water intubation or shock to the feet was paired with a distinctive taste. The procedure with each UCS consisted of three phases - pretraining preference tests, training trials and post-training preference tests. In the shock experiment, subjects were shocked for consumption of their preferred solution while consumption of their nonpreferred solution was not punished. In the sickness experiment, experimental subjects either received a salt water stomach load followed by access to the preferred solution on one day and a plain water stomach load followed by access to the nonpreferred solution on the next day, or vice versa. This two day cycle was repeated five times. In both experiments preference tests for the two solutions were carried out both before and after training. For the subjects in the sickness experiment and those in one subgroup of the shock experiment, pre- and post-training preference tests consisted of a two bottle test with the two solutions presented in the same place (i.e., a foods-together technique). Another subgroup of the shock experiment received pretraining preference tests which were identical to those just described, but post-tests consisted of presenting the two bottles at the same time but in different places, as they had been in training (i.e., a foods-apart technique).

The results of the Braveman and Capretta (1965) study

showed that salt water intubation was effective in producing food preference modifications under a foods-together testing technique. Shocked animals, tested under the same conditions which they had been trained under (foods-apart), did show the preference change whereas those tested with the foods-together technique showed no change. This indicated that with shock as the UCS it was the place cues (external stimuli) that were important to the association, a finding substantiated by Garcia and Koelling (1966) and by Garcia, Kovner and Green (1970). At the same time, Braveman and Capretta's sickness experiment showed that the pairing of taste cues (internal cues) with sickness led to the formation of an aversion that was more pronounced than that produced by pairing shock with taste under comparable test conditions, a finding also substantiated by Garcia and Koelling (1966) and by Garcia, Kovner and Green (1970). Moreover, both of these findings by Braveman and Capretta (1965) were in agreement with Capretta's earlier finding (1961) that the source of the physical discomfort is important in conditioned food preference modifications.

Since then the principle of stimulus relevance and variations of it have been used to account for long delay learning, i.e., the associations are "relevant" for the animal and hence can be formed even if the delay between the stimuli is several minutes or hours (Revusky, 1971).

Relevance principles have also been used to explain poison-based avoidance learning with as few as one training trial, i.e., the association is more relevant to the animal, in terms of survival for instance, than more arbitrary ones such as associating barpressing with food (Revusky, 1971). The principle of stimulus relevance has, moreover, become the central facet of Revusky's (1971) concurrent interference theory, one of the more important theoretical explanations of animal memory, which states that the events between the occurrence of CS and UCS interfere with the association between CS and UCS only if they are similar to, or relevant to, either CS or UCS. However, if CS and UCS are highly relevant to each other, the chance of interference from intervening events will be diminished. Hence the principle of stimulus relevance can be seen to be an integral part of concurrent interference theory which in turn can account for long delay and one trial learning.

In light of the contemporary importance of relevance as an explanatory principle, both in its own right and as a central part of the principle of concurrent interference, it is paradoxical that Capretta (1961) and Braveman and Capretta (1965) did not employ a long CS - UCS interval or one training trial in their studies. For example Capretta (1961) used nine aversive training trials and Braveman and Capretta (1965), five aversive training trials. Also both of these

studies used the shortest possible CS - UCS interval, i.e., the UCS immediately preceded the CS so that sickness was contiguous with consumption of the CS. It was with these exceptions in mind that a series of studies (Andrews & Braveman, unpub. data) were carried out in an attempt to find whether long delay learning was possible with the technique used by Braveman and Capretta (1965).

The two studies followed the procedure of Braveman and Capretta (1965) except that three CS - UCS pairings and longer CS - UCS intervals were used. In study 1 the CS - UCS delays were one and five hours, while in study 2, they were one and seven hours. Study 2 also differed from the first study in that subjects were water deprived during pre- and post-training preference tests. During training in study 2 and during all phases of study 1, subjects were not water deprived. The results of the two studies are shown in Figure 1 and indicated that long delay learning could in fact occur with intubated saline as the UCS, but the conditions of the studies did not allow an evaluation of the aversion after only one training trial. Also, the results were equivocal with respect to the finding of a delay of reinforcement gradient. That is, while the findings of study 1 showed that aversions were stronger with the shorter CS - UCS interval, no evidence for a gradient was found in the second study, even though a longer CS - UCS interval was employed.

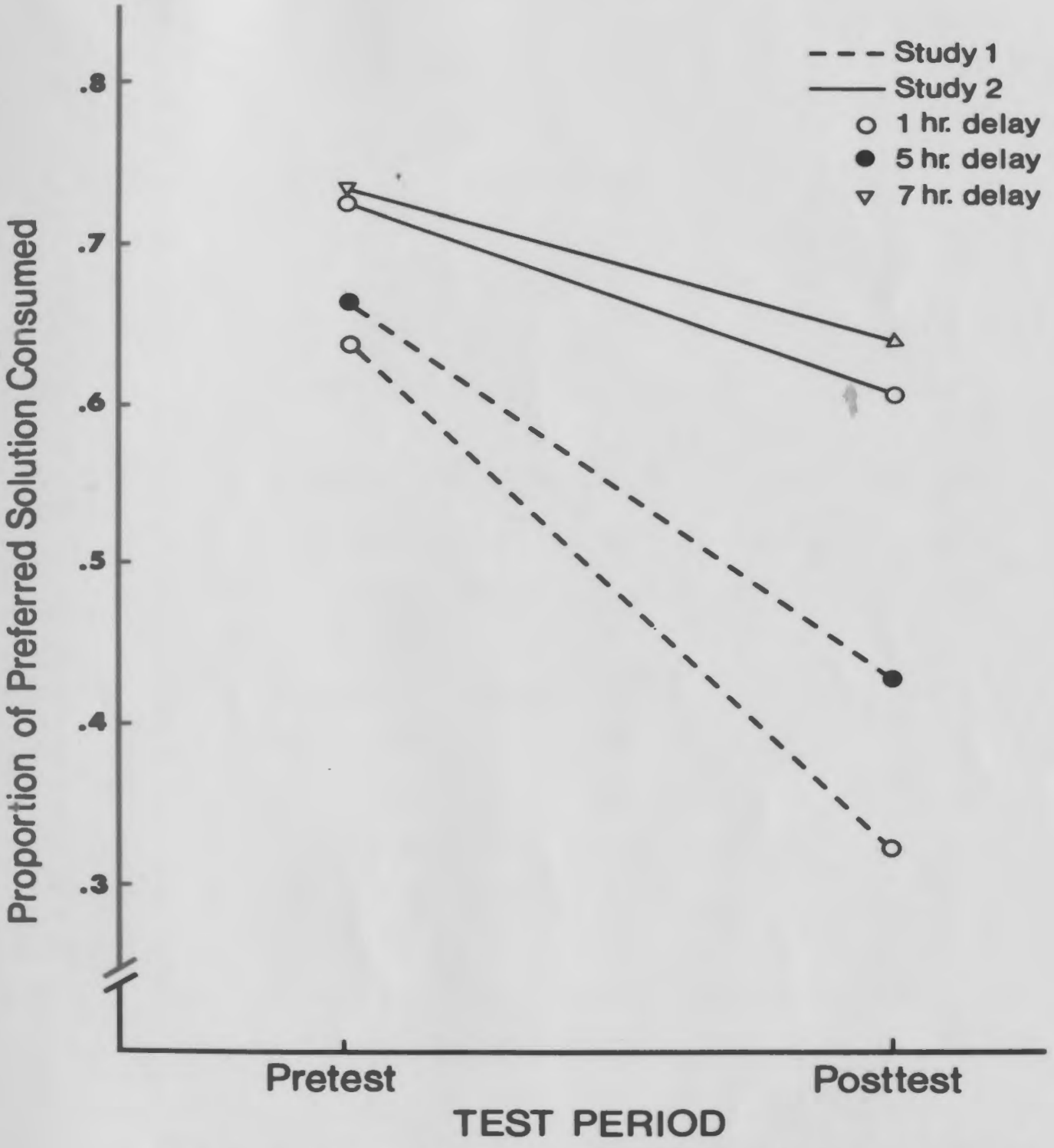

Fig. 1 Proportion of Preferred Solution Consumed in Pilot Studies 1 and 2

The inability to find a clear-cut gradient of reinforcement was of particular importance since the occurrence of these gradients for learned taste aversions has been one of the strongest arguments in support of the view that long delay aversions do not violate the accepted ideas of learning becoming poorer at longer CS - UCS delays. Also, delay of reinforcement gradients have been shown to be a general characteristic of other toxins (e.g., apomorphine - - Garcia, Ervin & Koelling, 1966; Green, 1969; cyclophosphamide - - Wright, Foshee & McCleary, 1971; lithium chloride - - Kalat & Rozin, 1971; Nachman, 1970 a; Rozin & Ree, 1972; thiamine deficiency - - Garcia, Ervin, Yorke & Koelling, 1967; irradiation - - Revusky, 1968; Smith & Roll, 1967). Thus, on the one hand, the salt water intubation method has been the basis for a learning interpretation of poison-based aversions, but on the other hand an equivocal delay of reinforcement gradient was obtained when this technique was used at CS - UCS delays of up to seven hours.

Among the possible factors which may have caused the failure to find a clear gradient in the two pilot studies are floor effects from the three training trials or the use of CS - UCS intervals which were not long enough. Either of these factors could have counteracted a delay of reinforcement gradient. Floor effects, in general, refer to the situation where differential effects are obscured by minimal

consumption of the test solution by different groups of experimental animals. This leads to a situation where it is not possible to detect significant differences between the groups (Revusky & Garcia, 1970). There are two ways that this could have happened in the pilot studies. First, it could have occurred as a result of several CS - UCS pairings having been given before any post-training preference tests took place. Toxins that might have produced aversions of different strengths after one training trial may have appeared to be of equal strength after several training trials since the stronger condition (i.e., the shorter CS - UCS delay) could have produced a maximal effect after one or two trials, and hence not shown any improvement with further training trials. The weaker condition, meanwhile, could have also reached maximal effectiveness by three trials, and would then have appeared as effective as the shorter delay by the time preference tests were begun. Secondly, gradient effects could have been absent from the data because of the use of CS - UCS intervals that were of insufficient duration. That is, a UCS which would show a delay of reinforcement gradient with CS - UCS intervals of up to eight or ten hours might not show the gradient at all if the CS - UCS intervals are of only short duration. In the case of the two pilot studies either or both of these possibilities could have counteracted the occurrence of a delay of

reinforcement gradient. Perhaps a gradient would have occurred if only one CS - UCS pairing had been used, or if longer CS - UCS intervals had been employed.

The purpose of the present experiment was to investigate the use of one such CS - UCS pairing and longer CS - UCS intervals. Animals were intubated once with saline after consumption of a saccharin solution in order to assess the reinforcement gradient for this toxin. The CS - UCS intervals of 0.5, 1.5, 4.5, 13.5 and 24.0 hr. were factorially combined with saline concentrations of 0.9%, 2.7%, 8.1% and 12.15% (w/v). (The 0.9%, that is isotonic, saline groups served as control groups.) Parametric studies of this kind do not seem to have been very popular in poison-based avoidance learning studies. In fact, only one study was found in the literature which also varied CS - UCS interval and toxin strength factorially. This was a study by Wright, Foshee and McCleary (1971) who examined the effects of cyclophosphamide as a toxin at various dosage levels and CS - UCS delays. The dosage levels and delays used were 25, 50 and 75 mg/kg of body weight of cyclophosphamide at delays of 30, 75 and 120 min. Three pairings of drug and saccharin flavored water were followed by three preference tests with saccharin alone. The subjects were on a 24 hr. water deprivation schedule throughout training and testing. The results of the post-training preference tests showed that drug-dose level,

CS - UCS delay, number of trials and the drug-dose x trials interaction were significant. In short, they found that a gradient of reinforcement effectiveness did exist - the longer delays led to less marked aversions than did short delays and higher dosage levels of the UCS led to stronger aversions. In the present study, therefore, it was expected that in general higher concentrations would produce stronger aversions than lower ones, and that short CS - UCS delays would lead to stronger aversions than longer ones. It was also expected that higher concentrations would be effective at longer CS - UCS delays than lower concentrations would.

Method

Subjects

The Ss were 160 experimentally naive male albino rats, 90 days old at the start of the experiment. These were obtained from the Canadian Breeding Laboratories and were maintained in individual cages on ad lib. food throughout the experiment. Six Ss died during the experiment and were replaced in later replications so that there were eight Ss in each experimental and control group.

Apparatus

Testing and training were carried out in the animal's home cage. Special containers were clipped to the side of the cage for the presentation of tap water or the saccharin solution. The saccharin solution used was a 0.5% (w/v) concentration of sodium saccharin. The saline concentrations used for intubation were 0.9, 2.7, 8.1 and 12.15% (w/v) solutions of sodium chloride in water.

Saline intubation was accomplished using a small polyethylene catheter (I.D. = .034 in., O.D. = .050 in.), a mouth brace cut from a No. 6 one hole hard rubber stopper, and a syringe in a metal stand.

Procedure

Upon arrival at the lab, all Ss were placed in individual cages with ad lib food and water for three days. They were

then deprived of water for 48 hr. On the third day they were given water for three hours, and on the fourth day they were placed on a 10 - min. a day drinking schedule. Water intake during the 10 - min. drinking periods was recorded to the nearest 0.5 ml. for six days when all Ss reached a stable level of consumption. On the training day Ss, randomly assigned to one of 20 groups, received a 10 - min. drinking period with saccharin after 24 hr. water deprivation. Then, after a CS - UCS interval of 0.5, 1.5, 4.5, 13.5 or 24.0 hr., groups of Ss were intubated with a 1.5% body weight dose of 0.9, 2.7, 8.1 or 12.15% saline. Two hours after saline intubation all Ss received water for six hours to facilitate recovery from the saline-induced sickness. Forty-eight hours after saccharin presentation all Ss were returned to the 10 - min. a day water schedule.

The intubation procedure used on training day was modelled after that of Capretta (1962) and Braveman and Capretta (1965). Briefly, intubation was accomplished by holding S's mouth open with the rubber brace and inserting the catheter directly into the stomach. The end of the catheter was first dipped in water to encourage the rat to swallow it. Once the catheter was in the rat's stomach, the saline was injected slowly by means of the syringe attached to the catheter.

Five post-training preference tests began three days after the Ss had been returned to the 10 - min. a day drinking

schedule. These tests involved presenting the saccharin solution to Ss for 10 min. on every second day. The amount of saccharin or water consumed on each day was recorded to the nearest 0.5 ml. On the alternate days Ss received water during the 10 - min. test.

Results

The results of the experiment were analyzed in two parts. First, the amount of saccharin consumed prior to sickness was analyzed to ensure that all 20 groups initially consumed the same amount of saccharin. Second, in order to assess the combined effects of CS - UCS interval and dosage, the amount of saccharin consumed on each post-training preference test was analyzed in a repeated measures analysis of variance. Individual comparisons were also carried out to determine which conditions had produced significant aversions, and whether gradients of UCS effectiveness had occurred.

Pretraining Tests

The mean amount of saccharin consumed by each group before training is presented in Table 1. A 5 x 4 (Delay x Concentration) analysis of variance (Winer, 1962) on the amount of saccharin consumed is summarized in Table 2 and shows that there were no significant differences among groups in their consumption of saccharin prior to training. Thus, it is apparent that before the experimental treatment was carried out there were no differences in the saccharin consumption of the various experimental and control groups.

Table 1

Mean Saccharin Consumption and Standard Deviations for all Groups on Training Day (in ml.)

Concentration of Saline		CS - UCS Delay 0.5hr.	1.5hr.	4.5hr.	13.5hr.	24.0hr.
0.9%	$\bar{X}$	5.69	5.0	4.56	5.38	5.25
	S.D.	2.71	1.96	1.66	2.03	1.41
2.7%	$\bar{X}$	4.5	5.31	4.63	4.81	6.06
	S.D.	1.93	1.96	1.83	1.56	1.29
8.1%	$\bar{X}$	6.0	5.94	5.44	5.63	5.44
	S.D.	2.42	2.46	1.64	1.46	2.64
12.15%	$\bar{X}$	4.81	5.94	4.19	4.94	4.13
	S.D.	1.39	2.44	1.53	1.99	0.95

Table 2

Analysis of Variance for

Pretraining Consumption of Saccharin

Source	SS	df	MS	F
Delay (D)	11.79	4	2.95	.80*
Concentration (C)	16.63	3	5.54	1.50*
D x C	26.45	12	2.20	.60*
Error	516.88	140	3.70	-

* Not Significant

Post-training Tests

The results of the post-training preference tests are presented in Table 3. This table includes the 0.9% saline groups. However, since the isotonic groups were control groups their data were not included in the main analysis of variance. The reason for excluding their data was that, in contrast to subjects' in the 2.7%, 8.1% and 12.15% groups, subjects in the 0.9% groups did not decrease their saccharin intake at any delay condition following training (see Tables 1 and 3). Hence including these isotonic groups in the main analysis would probably have inflated any effects that might have been present. To be certain that 0.9% led to no differential effects across CS - UCS delays on preference days the saccharin intake of subjects in these 0.9% groups was analyzed separately in a 5 x 5 (Delay x Preference Test) repeated measures analysis of variance (Winer, 1962). A summary of this analysis is presented in Table 4 and shows that there were no differential effects from delays. The only significant effect was a gradual increase in consumption over preference days which reflects only the gradual reduction of neophobia and certainly does not indicate any aversive effects from the isotonic saline.

Table 3

Mean Saccharin Consumption and Standard Deviations for all Groups on Post-training Preference Test Days 1 to 5 (in ml.)

Day	Saline Concentration		CS - UCS Delay (in hr.) 0.5	1.5	4.5	13.5	24.0
1	0.9%	$\bar{X}$	9.81	10.56	9.94	10.63	9.94
		S.D.	3.38	4.39	2.37	3.00	2.53
	2.7%	$\bar{X}$	6.38	5.94	6.19	9.88	14.00
		S.D.	3.70	2.90	1.71	4.08	2.09
	8.1%	$\bar{X}$	2.81	5.13	5.31	9.19	11.38
		S.D.	1.56	1.77	2.99	3.47	6.26
	12.15%	$\bar{X}$	1.56	2.81	3.94	7.63	9.00
		S.D.	1.27	1.75	2.61	2.03	3.88
2	0.9%	$\bar{X}$	13.31	15.94	12.69	14.00	15.63
		S.D.	3.33	5.18	2.84	4.50	4.56
	2.7%	$\bar{X}$	10.81	10.00	9.44	13.25	16.00
		S.D.	5.24	2.90	2.47	3.01	2.05
	8.1%	$\bar{X}$	5.19	9.19	8.25	13.75	15.44
		S.D.	3.32	4.62	3.66	3.40	5.13
	12.15%	$\bar{X}$	2.63	4.75	6.06	10.56	14.75
		S.D.	2.37	3.19	4.21	2.13	2.33

Table 3 (continued)

Day	Saline Concentration		CS - UCS Delay (in hr.) 0.5	1.5	4.5	13.5	24.0
3	0.9%	$\bar{X}$	13.81	16.19	12.69	14.56	15.94
		S.D.	2.85	4.55	2.88	3.66	3.91
	2.7%	$\bar{X}$	13.88	15.31	12.56	14.69	16.31
		S.D.	5.24	2.80	3.42	2.56	3.27
	8.1%	$\bar{X}$	8.69	11.31	11.75	16.56	15.56
		S.D.	4.38	3.71	4.83	3.91	5.72
	12.15%	$\bar{X}$	5.44	8.81	8.75	13.63	16.63
		S.D.	4.24	5.36	5.13	2.55	2.52
4	0.9%	$\bar{X}$	15.56	15.81	16.06	16.81	17.13
		S.D.	2.71	2.24	3.57	3.36	3.47
	2.7%	$\bar{X}$	16.44	17.38	12.19	17.19	16.13
		S.D.	3.02	2.89	2.33	2.99	2.47
	8.1%	$\bar{X}$	12.06	13.75	13.56	17.56	19.06
		S.D.	3.89	3.41	3.90	3.39	4.47
	12.15%	$\bar{X}$	9.38	12.69	11.94	15.31	17.75
		S.D.	6.02	6.38	5.17	2.56	1.56

Table 3 (continued)

Day	Saline Concentration		CS - UCS Delay (in hr.) 0.5	1.5	4.5	13.5	24.0
5	0.9%	$\bar{X}$	16.13	17.88	16.00	18.13	18.38
		S.D.	1.87	3.14	2.44	3.49	3.73
	2.7%	$\bar{X}$	17.56	16.06	14.06	16.69	18.38
		S.D.	2.31	3.65	3.02	2.22	1.71
	8.1%	$\bar{X}$	15.06	14.31	15.31	16.94	19.13
		S.D.	3.32	2.15	3.39	3.01	4.18
	12.15%	$\bar{X}$	12.38	15.94	15.56	16.06	17.75
		S.D.	6.24	4.97	6.14	2.40	1.75

Table 4

Analysis of Variance on Saccharin Intake by Isotonic Saline Groups on Post-training Preference Tests

Source	SS	df	MS	F
Delay (D)	126.38	4	31.59	0.79*
Error	1407.01	35	40.20	-
Preference day (P)	1189.69	4	297.42	61.22**
D x P	68.00	16	4.25	0.88*
P x Subj w. groups	680.12	140	4.86	-

* Not significant

** $p < .001$

The results of a 5 x 3 x 5 (Delay x Concentration x Preference Test) repeated measures analysis of variance (Winer, 1962) on the amount of saccharin consumed by experimental animals on the five post-training preference test days are summarized in Table 5. This analysis shows that all main effects were significant. In addition, the Delay x Preference Test, Concentration x Preference Test and Delay x Concentration x Preference Test interactions were also significant showing that the groups drank differing amounts of saccharin on the test days.

In order to assess the precise differences among the groups and to discover exactly what between day differences there were in the aversions, comparisons, using Dunnett's test for making multiple comparisons with the same control (Edwards, 1960), were made between the amount consumed by each experimental group and the corresponding control group which had received isotonic saline. An aversion was defined as saccharin consumption by an experimental group which was significantly lower than consumption by its respective control group. For example, the daily consumption on each preference test of the 2.7% - 0.5 hr., 8.1% - 0.5 hr. and 12.15% - 0.5 hr. groups were all compared with that of the 0.9% - 0.5 hr. group. The mean consumption for each group on each of the five preference test days is shown in Figures 2, 3, 4, 5 and 6, respectively, which indicate the CS - UCS delays actually used as well as standard scale values, and the results of the

Table 5

Analysis of Variance for Saccharin Intake by Experimental Groups on Post-training Preference Tests

Source	SS	df	MS	F
Delay (D)	3517.19	4	879.30	20.53 ****
Concentration (C)	910.63	2	455.31	10.63 ****
D x C	527.31	8	65.91	1.54 *
Error	4497.69	105	42.84	-
Preference Test (P)	6800.19	4	1700.05	283.43 ****
D x P	421.81	16	26.36	4.40 ****
C x P	139.06	8	17.38	2.90 ***
D x C x P	296.13	32	9.25	1.54 **
P x Subj w. groups	2519.25	420	5.99	-

* Not significant

** $p < .05$

*** $p < .005$

**** $p < .001$

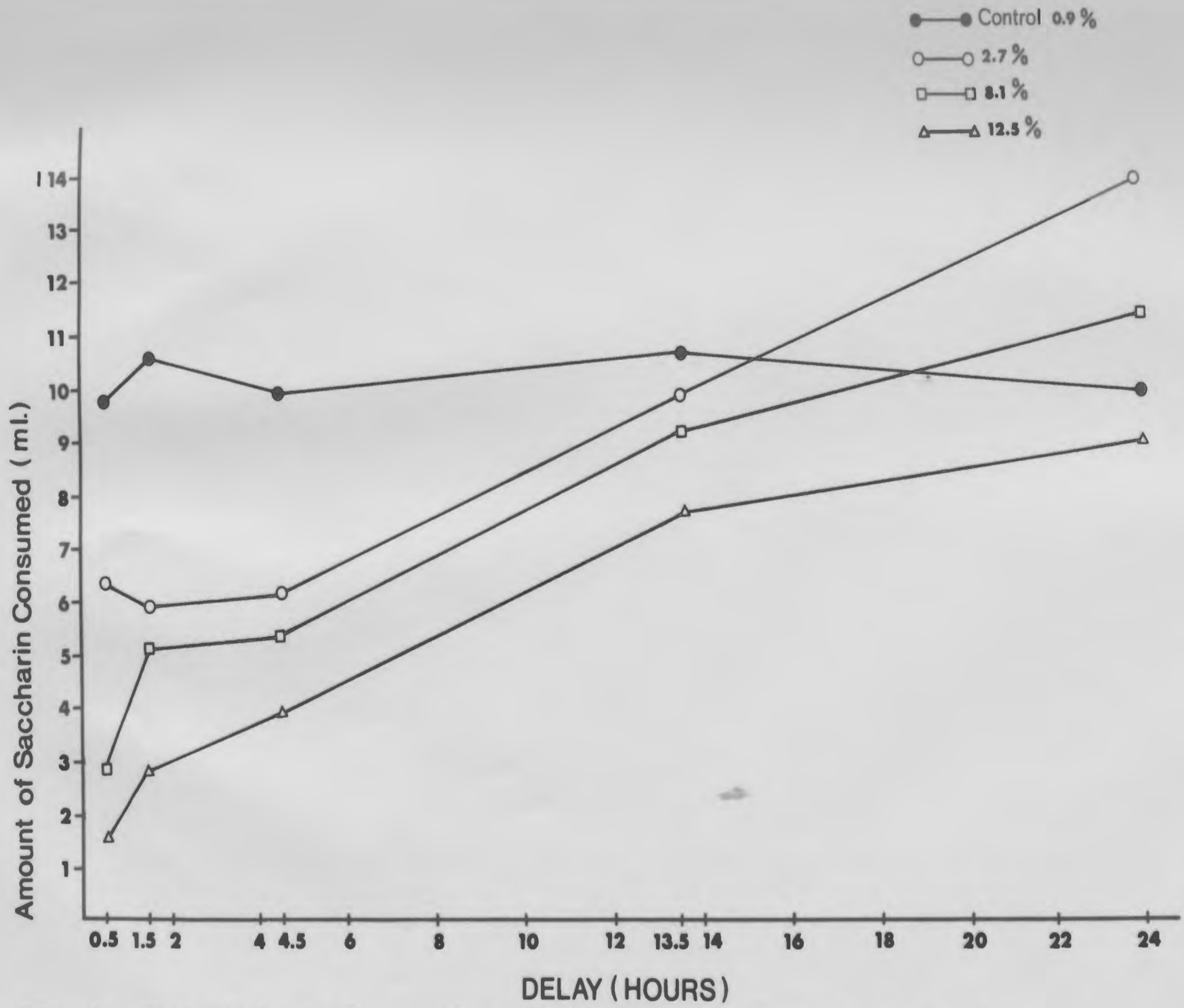

Fig. 2. Mean Saccharin Consumption of Subjects on Preference Test Day 1

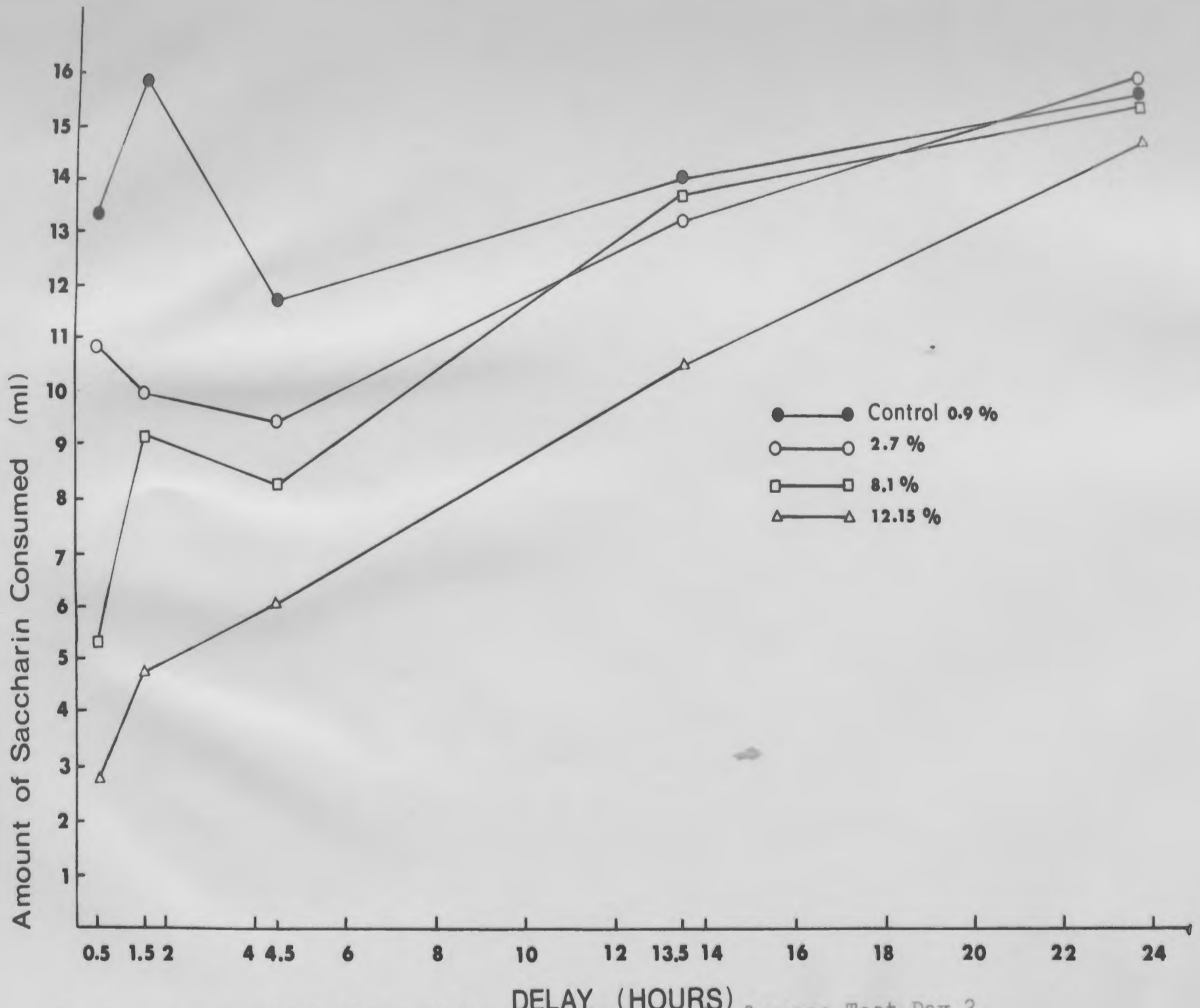

Fig. 3. Mean Saccharin Consumption of Subjects on Preference Test Day 2.

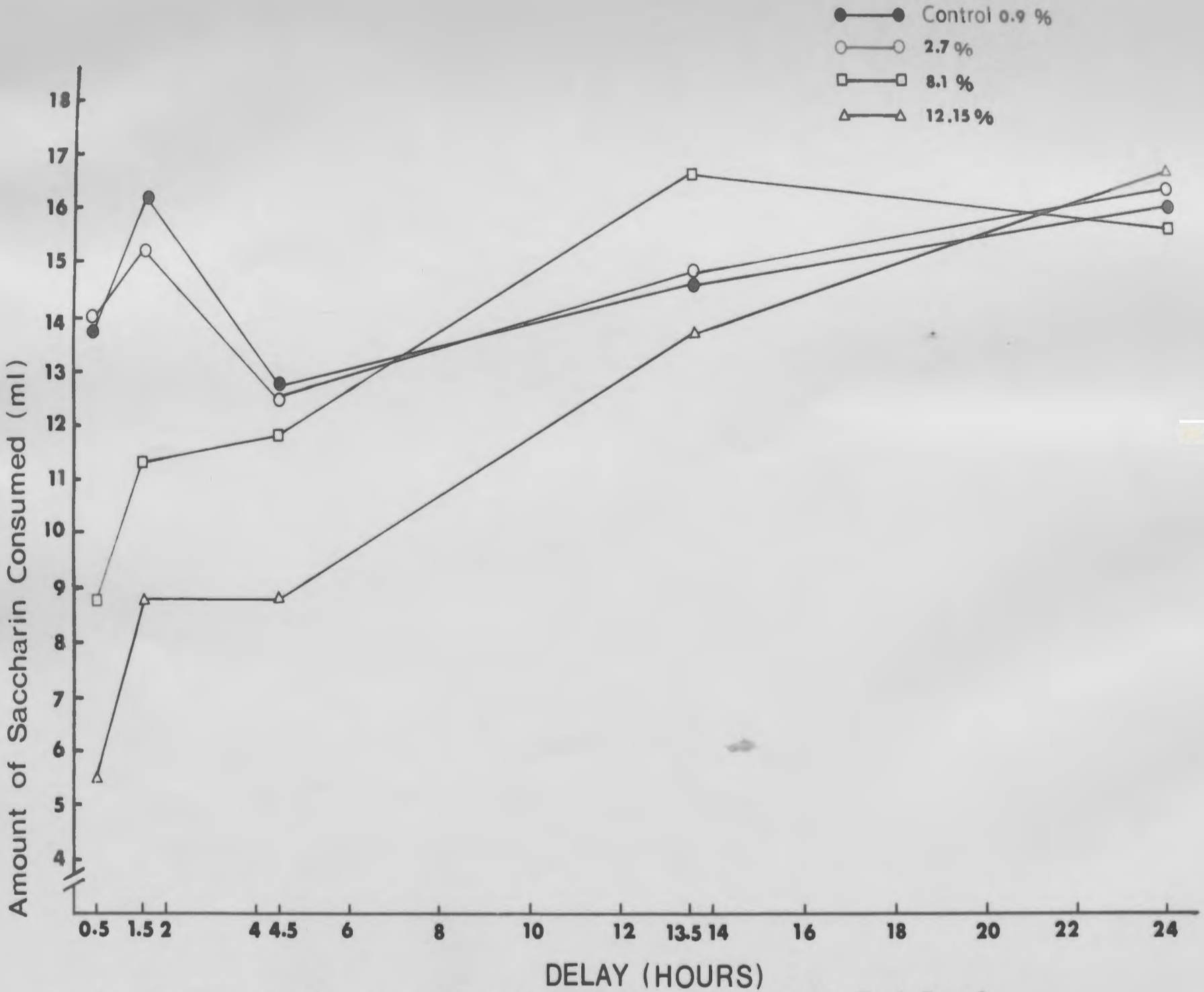

Fig. 4. Mean Saccharin Consumption of Subjects on Preference Test Day 3

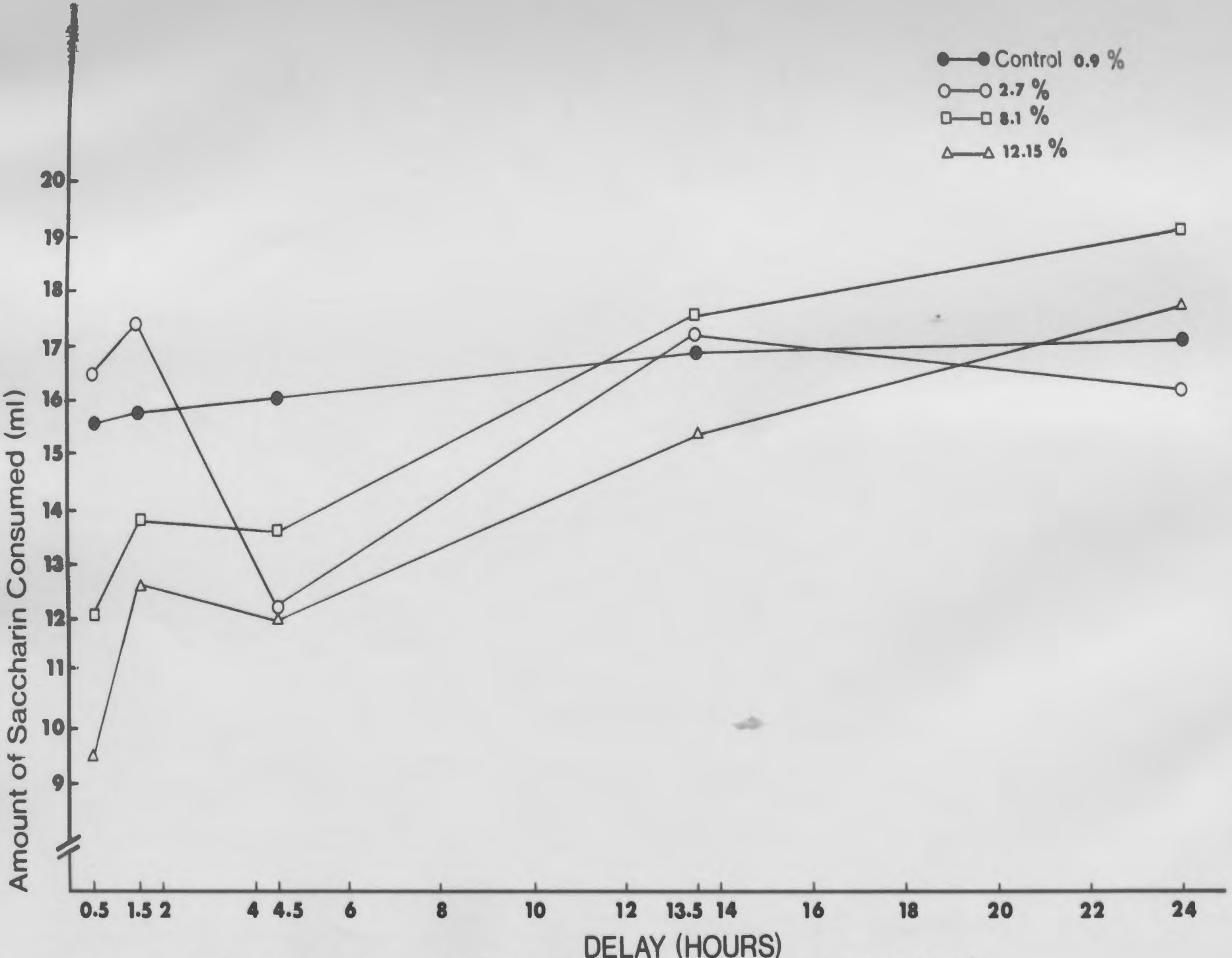

Fig. 5. Mean Saccharin Consumption of Subjects on Preference Test Day 4

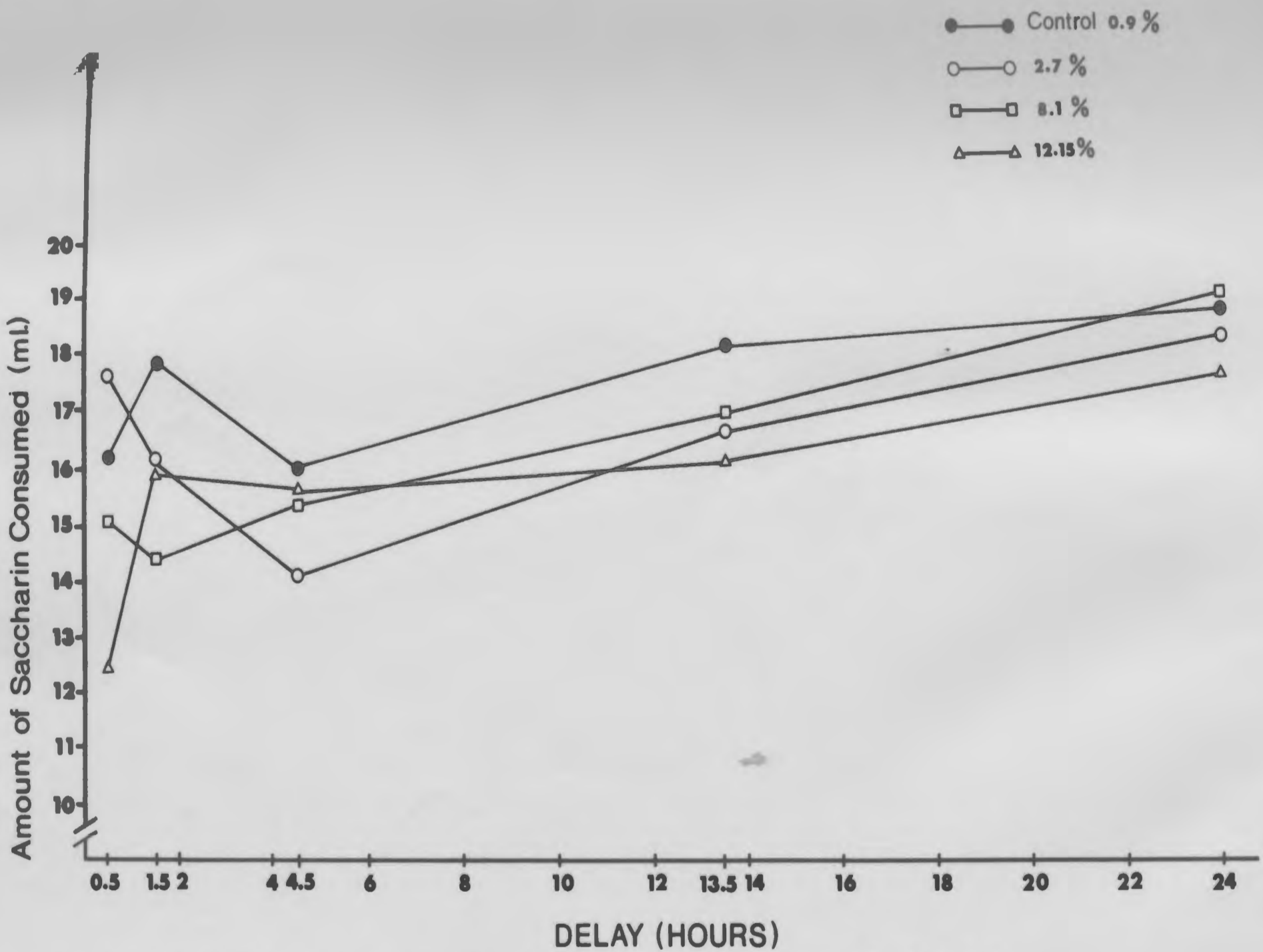

Fig. 6. Mean Saccharin Consumption of Subjects on Preference Test Day 5

Dunnett comparisons for each delay condition and preference test day are shown in Table 6 (see Table B, Appendix, for critical values in Dunnett comparisons).

Table 6 shows that, when differences between experimental and control subjects are considered across preference test days, gradient effects were indeed present. When the CS - UCS interval was extended to 13.5 hr., UCS's which were effective in producing aversions at shorter intervals were no longer effective. There were also gradients in the effectiveness of different concentrations. That is, the 12.15% aversions took longer to extinguish (i.e., show no difference in intake from the 0.9% group's intake) than the lower concentrations. It should also be noted that no experimental condition led to an enhancement of saccharin consumption.

More specifically, the Dunnett tests revealed that only the three experimental concentrations at CS - UCS delays of 0.5 hr., 1.5 hr. and 4.5 hr. showed significant aversions on Preference Day 1. No delay longer than 4.5 hr. produced significant effects with any of the saline concentrations. It can be seen from Figure 2 that all experimental groups at delays of 4.5 hr. or less consumed considerably less saccharin than their respective control groups while groups at delays longer than 4.5 hr. did not. In other words, aversions decreased in strength with increased CS - UCS interval and decreased saline concentration.

On Test Day 2, only seven of the nine groups that showed significant aversions on Test Day 1 still showed a

Table 6

Comparisons of all Experimental Groups to Control Groups at all CS - UCS Intervals on all Post-training Preference Days

Day	Saline Concentration	CS - UCS Delay (in hr.) 0.5	1.5	4.5	13.5	24.0
1	2.7%	*	**	**	-	-
	8.1%	**	**	**	-	-
	12.15%	**	**	**	-	-
2	2.7%	-	**	-	-	-
	8.1%	**	**	**	-	-
	12.15%	**	**	**	-	-
3	2.7%	-	-	-	-	-
	8.1%	*	*	-	-	-
	12.15%	**	**	-	-	-
4	2.7%	-	-	-	-	-
	8.1%	-	-	-	-	-
	12.15%	**	-	*	-	-
5	2.7%	-	-	-	-	-
	8.1%	-	-	-	-	-
	12.15%	-	-	-	-	-

* $p < .05$

** $p < .01$

significantly lower consumption than their control groups. The 2.7% - 0.5 hr. and 2.7% - 4.5 hr. groups no longer consumed less than their isotonic control groups. The 2.7% - 1.5 hr. group, however, still showed an aversion, which is not in line with the principle that the effectiveness of the UCS decreases as the CS - UCS interval increases since a group with the same saline concentration but a shorter delay (2.7% - 0.5 hr.) did not show an aversion on Day 2. Inspection of Table 3 shows that the mean for the 2.7% - 1.5 hr. group was not out of line with the other 2.7% groups. Thus it appears that the aversion found for the 2.7% - 1.5 hr. group probably occurred as the result of a sharp, and unexplained, rise in consumption by the 0.9% -1.5 hr. group, the control group for the 2.7% - 1.5 hr. group. This fluctuation was probably caused by sampling error and, therefore, is not a serious exception to the orderliness of the results.

On Test Day 3, none of the 4.5 hr. delay groups showed aversions, nor did the 2.7% - 1.5 hr. group. Only animals who had been trained with the two stronger concentrations, 8.1% and 12.15%, and at the two shortest delays, 0.5 and 1.5 hr., were still consuming less saccharin than their isotonic control groups. By Preference Day 4 only two groups, the 12.15% - 0.5 hr. and the 12.15% - 4.5 hr. groups, still showed significant aversions to saccharin. This also is not what would be predicted if the UCS decreases in effectiveness

as the CS - UCS interval increases since the 12.15% - 1.5 hr. group did not show an aversion while the 12.15% - 4.5 hr. group did. However, as Figure 5 shows, the consumption of these two groups, the 12.15% - 1.5 hr. and the 12.15% - 4.5 hr. groups, differed very little, with the result that the difference between the 12.15% - 4.5 hr. group and its control group was marginally significant while that between the 12.15% - 1.5 hr. group and its control group was not. Finally, on Preference Day 5, no groups differed from the isotonic groups in their saccharin consumption. That is, by the fifth preference day, all aversive effects of the UCS (as measured by lower saccharin consumption) had been extinguished.

The results of the Dunnett tests show a regular pattern with respect to the extinction rate of the aversions. That is, the nine aversions produced on Day 1 showed a gradient of effectiveness in terms of how well they resisted extinction during the subsequent preference tests. The aversion of the weakest saline concentration at the longest effective delay was the first to extinguish (i.e., the 2.7% - 4.5 hr. group). Next, all 4.5 hr. delay aversions extinguished, as did all 2.7% aversions. Then all 8.1% groups and 1.5 hr. groups ceased to be effective in maintaining their aversions. Finally the aversion from the strongest concentration at the shortest delay (i.e., 12.15% - 0.5 hr.)

extinguished. In other words, the aversion produced by the weakest concentration at the longest effective delay extinguished fastest and the aversion produced by the strongest concentration at the shortest effective delay extinguished slowest, while aversions produced by other combinations of concentration and delay were intermediate in their extinction rates.

Although it was clear from the Dunnett tests that a gradient of effectiveness did exist between preference days in terms of the extinction rates of the aversions, it was not clear whether such a gradient also was present within any of the preference test days. Therefore individual *t* tests (Ferguson, 1966) were made on the data for Preference Test Day 1. Comparisons were made on only the first test day since the results of the Dunnett's tests suggested that the differences would be most apparent then. Delay and concentration effects were considered separately since in the overall analysis, summarized in Table 5, the Delay x Concentration interaction was not significant. Even when considered over extinction days the Delay x Concentration interaction was only marginally significant. Hence, within a single preference test day, the effects of delay and saline concentration could be considered independently.

To look at delay effects, the experimental saline groups at each delay were pooled and compared with the isotonic

group for that delay. The result of this grouping is shown in Figure 7 (see Table 9, Appendix, for t values). The t tests revealed a pattern that was similar to the findings of the Dunnett tests for Preference Test Day 1. Intubated saline led to a decrease in saccharin consumption at CS - UCS intervals of 0.5 hr. ($p < .0001$), 1.5 hr. ($p < .01$) and 4.5 hr. ($p < .0001$). Intubated saline did not produce any decrease in saccharin consumption at intervals of 13.5 or 24.0 hr. Newman - Keuls comparisons among the means for the three groups which showed aversions revealed that there were no differences in consumption. Thus the delay gradient for CS - UCS delay is a two-point gradient on Test Day 1 - - delays up to 4.5 hr. were effective and those of 13.5 and 24 hr. were not.

The concentration effects were assessed by pooling the data for each saline concentration over CS - UCS intervals. Comparisons were made between each experimental group and the pooled isotonic control group. The result of this pooling of data is shown in Figure 8 (see Table 10, Appendix, for t values). The t tests here showed that all experimental saline groups were effective in producing conditioned aversions to saccharin ($p < .05$, $p < .0001$ and $p < .00001$ for 2.7, 8.1 and 12.15% respectively). Newman - Keuls comparisons among the three means showed that there was some grading in the effectiveness of the experimental saline concentrations.

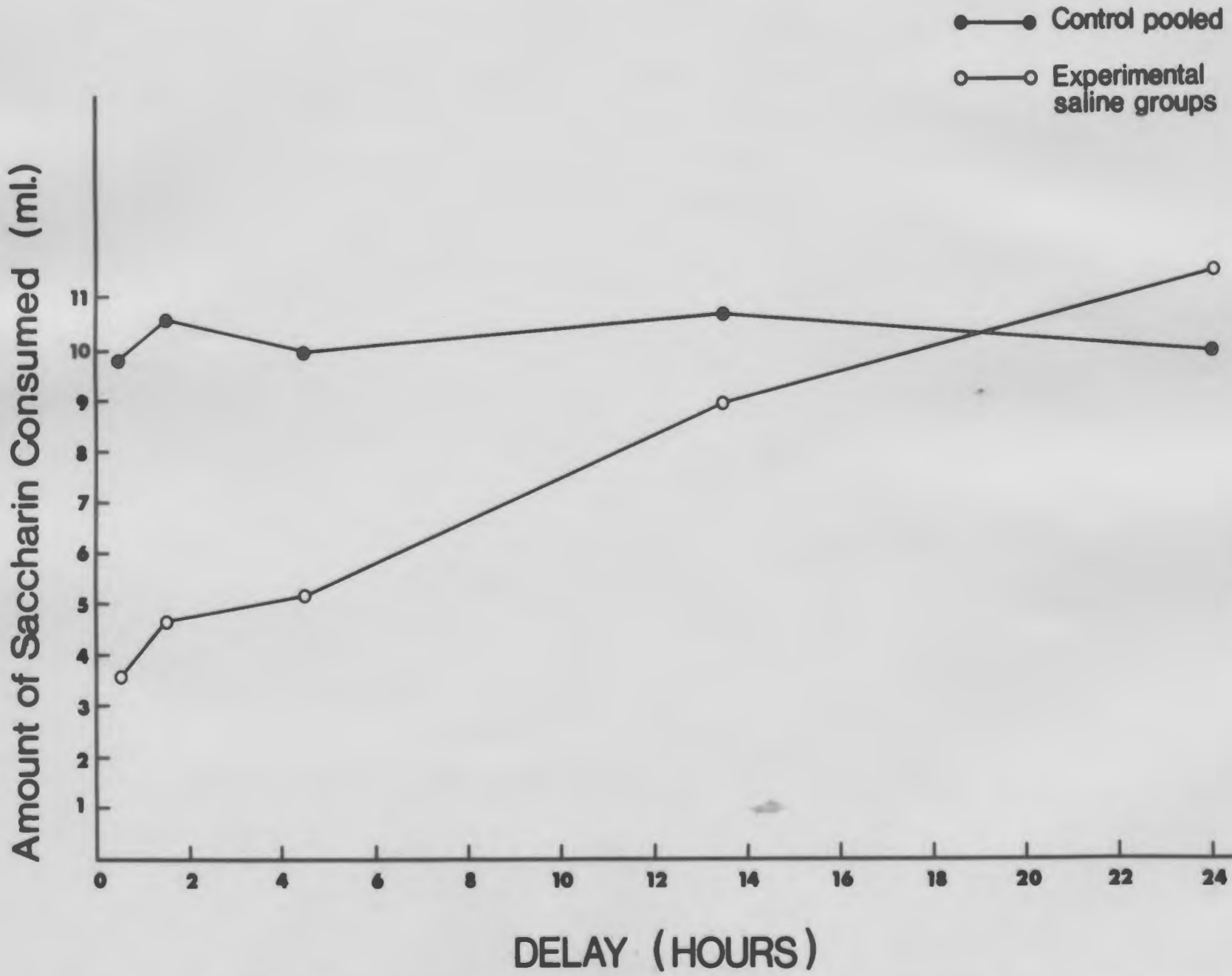

Fig. 7. Mean Saccharin Consumption of Pooled Experimental Saline Groups on Preference Test Day 1

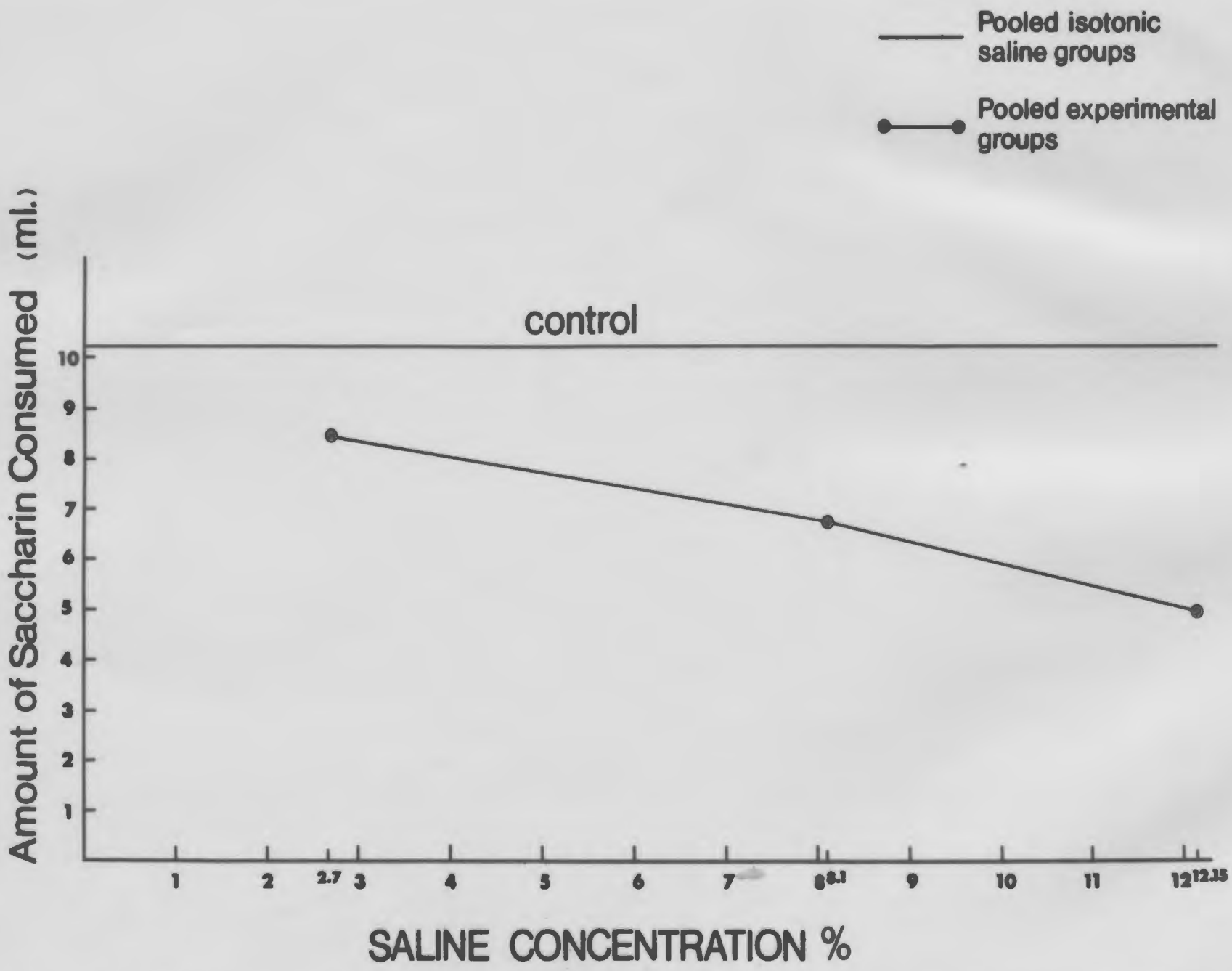

Fig. 8. Mean Saccharin Consumption of Pooled CS - UCS Delay Groups on Preference Test Day 1

The aversion produced by 12.15% saline was stronger than that produced by 2.7% saline. There was, however, no difference in the strengths of the aversions produced by 8.1 and 2.7% or in the strengths of the aversions produced by 8.1 and 12.15%.

The results of this analysis do not reveal as many differences in the initial strengths of the saccharin aversions as were reflected in the extinction rates of the various aversions (see Table 6). This difference may be accounted for in one of two ways. On the one hand the testing procedure may not have been sensitive enough to detect differences in the initial strengths of the aversions even though the procedure could reflect differences in the extinction rates of these aversions. On the other hand, part of the discrepancy may have been caused by floor effects in the data. However, since these same concentration and CS - UCS delay combinations extinguished at quite different rates, it seems unlikely that floor effects alone could have produced a maximal aversion in so many of the groups on Preference Day 1. Rather, it seems likely that a more sensitive preference test was needed. To keep the present research comparable with most other studies in poison-based avoidance learning, animals were tested here with a one bottle preference test when 24.0 hr. water-deprived. This produced conflict in the animals since their only

source of liquid on a test day was the saccharin solution which had been paired with sickness. The results were inflated saccharin consumption by the animals and hence few differences in the initial strengths of the aversions. The extinction rates did differ, however, since the animals with the strongest aversions took longer to overcome the conflict and increase their saccharin consumption any further from that of Preference Day 1. In other words, the relative strengths of the aversions were more reliably demonstrated by how long it took each group to extinguish its aversion.

Discussion

The main purposes of the present study were to see if aversions produced by intubated saline could be formed in one CS - UCS pairing at long CS - UCS intervals and to see if these saline induced aversions showed delay of reinforcement gradients similar to those found with other toxins. It is clear from the data that the use of intubated saline as a UCS was effective in producing one trial aversions over long CS - UCS intervals, and that delay of reinforcement effects were also present. Even the weakest saline concentration compared with isotonic saline led to significant aversions at delays up to 4.5 hr. on the first post-training preference test day, while no concentrations were effective in producing aversions at CS - UCS intervals of 13.5 hr. or longer. No experimental condition led to any enhancement of saccharin consumption. The strongest concentration used, 12.15%, led to almost complete rejection of the test solution by animals in the present experiment. This aversion, moreover, took four preference test days to extinguish at two of the CS - UCS delays used. Aversions created with the other concentrations were not as strong and extinguished sooner. Thus there can be little doubt as to the effectiveness of the intubated saline as a UCS. Moreover,

the findings of the study may be taken as further support for the generality of delay of reinforcement gradients in long delay poison-based avoidance learning.

The effectiveness of intubated saline in producing long delay poison-based aversions can be compared with the findings of representative studies using other toxins. For example, Green (1969) using three pairings of apomorphine as a UCS and grape juice as a CS found that a delay of two hours did not produce as strong an aversion as a delay of five minutes did. Nachman (1970a) used one pairing of lithium chloride as UCS and saccharin as CS at CS - UCS delays of 1 min., 15 min., 60 min., 4 hr., 8 hr. or 12 hr. He found that the amount of aversion was directly related to the sickness delay interval but that animals at all delay conditions were consuming significantly less saccharin than a control group injected with isotonic saline consumed. Smith and Roll (1967) used one pairing of irradiation as UCS and saccharin as the CS at CS - UCS delays of 0.0, 0.5, 1.0, 2.0, 3.0, 6.0, 12.0 and 24.0 hr. They found a very clear gradient of effectiveness - the treatment produced significant aversions at delays up to 6.0 hr. The aversion at the 12.0 hr. delay, although reported by Smith and Roll as an aversion was actually not significant ($p < .07$). The 12 hr. animals did, however, consume less saccharin than the 24.0 hr. animals. From these studies it can be concluded that the

delay gradient found with saline is not unlike that found with other toxins. The saline aversion seems to be stronger than that from apomorphine, since the apomorphine aversion showed a decrease in effectiveness at a delay of two hours. However, as two hours was the longest delay used by Green (1969) in the apomorphine study a more complete comparison is not possible. The gradient found for lithium chloride by Nachman (1970a) is very similar to the saline gradient found here. By four or four and half hours, animals were consuming more than they did at the shorter delays, but were still showing a significant aversion. It is not possible though to determine where lithium ceases to be effective, since, in the Nachman study, it produced significant aversions at all delays tested (i.e., up to twelve hours). Radiation also produced a similar gradient to that shown by saline, and ceased to be effective at close to the same CS - UCS delay (i.e. at about twelve hours). In short, the saline intubation led to a delay of reinforcement gradient not unlike those found with other toxins in general shape and length.

The results of the present study can also be compared with the gradient found with one other toxin - cyclophosphamide - in a study by Wright, Foshee and McCleary (1971). This study was more similar to the present one than those already mentioned in that both UCS dosage and CS - UCS

interval were varied. Specifically, the study used three training trials with cyclophosphamide as the UCS, at dosage levels of 25, 50 and 75 mg/kg of body weight, and at CS - UCS intervals of 30, 75 and 120 min. Extinction was measured over three post-training preference tests. Direct comparisons between the gradient of the Wright et al (1971) study and that of the present one are only possible at CS - UCS delays of up to 2 hr. since that was the longest delay used by Wright et al. Within this limit, the cyclophosphamide gradient appears to have been steeper than the saline one since the Wright et al study did find a delay effect with delays as short as 2 hr. Delays of 4.5 hr. were necessary to observe a decrease in effectiveness with saline as the UCS. Also it is not possible to say just how long a gradient cyclophosphamide would produce as this was not tested in the Wright et al study.

One more factor about the saline gradients should be mentioned. On Preference Test Day 1 the gradients were of a very abrupt nature. With respect to concentration, a saline strength of 12.15% was more aversive than one of 2.7% but was equivalent in strength to one of 8.1%. In terms of delays between CS and UCS, delays of 0.5, 1.5 and 4.5 hr. produced aversions of equivalent strength while delays of 13.5 and 24.0 hr. produced no aversions. The delay at which intubated saline ceases to be effective must lie between CS - UCS intervals of 4.5 and 13.5 hr. but

its exact location cannot be determined from the present data. From the two studies mentioned which have used delays of 12 hr. or longer (Nachman, 1970 a; Smith & Roll, 1967) it can be predicted that consideration of intermediate delays should lead to aversions of intermediate strength. Hence, to see whether the concentrations of saline used in the present study differ in terms of the maximum interval at which each is effective, further research will need to look at CS - UCS delays between 4.5 and 13.5 hr.

One point of interest related to Capretta's (1961) and Braveman and Capretta's (1965) findings is the range of saline concentrations found to be nonlethal. Both studies used 10% saline, although neither states why this concentration was used. From the pilot work done to select the concentrations for the present study it is now quite clear that 10% is in fact fairly close to the upper limit of nonlethal doses. This follows from the fact that the pilot work here found that any dose over about 13% saline would be fatal in some, if not all, animals who had been on a water - deprivation schedule for two weeks.

Finally, the findings of the present experiment shed light on the outcome of the pilot studies and on why one of them produced a delay of reinforcement gradient while the other did not. In the present study, aversions with the 12.15% concentration were found at CS - UCS delays up to

4.5 hr. With only one training trial, but none were found at a delay of 13.5 hr. Moreover, the 12.15% aversion in the present study was actually showing a decrease in effectiveness by the 4.5 hr. delay. In pilot study 2, which was quite similar to the present study in terms of the deprivation conditions employed, 10% saline (with three training trials) produced no decrease in effectiveness at delays up to seven hours using a two bottle (i.e., more sensitive) test (see Dragoin, McCleary and McCleary, 1971). Thus it seems most probable that, since the main differences between the two studies were in the number of training trials, the failure of pilot study 2 to find a delay of reinforcement gradient was the result of floor effects generated by the number of CS - UCS pairings used. With only one training trial there is no reason to conclude that 10% saline would not produce a gradient of effectiveness lying between those for 8.1% and 12.15%. This implies that 10% saline under such conditions would show a decrease in effectiveness with increases in CS - UCS intervals up to seven hours.

Pilot study 1 was slightly different from pilot study 2 and from the present study in procedure. In the first place delays of only up to five hours were used; secondly, subjects were not water deprived; and thirdly, in spite of using three trials, a gradient effect was found. Comparison of this pilot study with the results of the present experiment is difficult because of the difference

in deprivation conditions. However, since it seems probable that floor effects were present in pilot study 2, this fact may be used as a starting point to consider the difference between the results from the two pilot studies. The most likely explanation of the difference is in terms of the actual preference levels shown by the animals. Pilot study 2 animals, although given a two bottle preference test, could, like those of the present study, have been in a conflict situation when preference tested. Although they may have formed an aversion for the test solution, their thirst may have conflicted with the aversion during the preference test. Pilot study 1 animals, again with a two bottle test, faced no such dilemma because they were not water deprived. The result was that, in general, pilot study 1 animals showed much lower preferences for the test solution (see Figure 1). That is, the pilot study 1 one hour delay group showed a lower post-training preference for the test solution than did the pilot study 2 one hour delay group. This seems to indicate that a second reason for the failure of pilot study 2 to show a gradient may have been the forced extinction of the aversion in the pilot study 2 animals. Although the post-training preferences of the pilot study 2 animals were less than the pre-training preferences, the seven hour group could not show a weaker aversion than the one hour group because the one hour group was already showing a considerably higher preference than it would if it had not

been water-deprived.

This question of differences in deprivation is one which has since been further investigated by the author (Andrews and Braveman, unpub. data). It is a question that will be of importance in any attempt to apply poison-based aversions in such areas as aversion therapy (Revusky, in prep.) or pest control (Martin, in prep.). In such situations subjects would probably not be on a severe liquid deprivation schedule. The indication of pilot study 1 is that such a procedure will probably produce stronger aversions than when a deprivation procedure is used. This in turn implies that the present experiment might have produced stronger aversions and longer delay of reinforcement gradients if subjects had not been water-deprived. Hence, the absence of strict liquid intake control in the use of poison-based avoidance learning in alcohol aversion therapy or in pest control may be an asset rather than a disadvantage. However, in order to keep fairly close to the procedures of most poison-based avoidance learning studies, Capretta's in particular, a deprivation procedure was employed in the present experiment. The question is one, however, that may warrant further study in the future.

To summarize, the present experiment varied saline concentration (UCS) and CS - UCS delay factorially in a one trial poison-based avoidance learning situation. Results indicated that the main effects for concentration, delay and preference test day were all significant. There were

also significant Delay x Preference Test, Concentration x Preference Test and Delay x Concentration x Preference Test interactions. These findings were interpreted as meaning that one trial saline - induced aversions could be produced with saline concentrations of 2.7, 8.1 and 12.15% at CS - UCS intervals up to 4.5 hr. These aversions followed the usual learning relationships in such a way that the strength of aversion varied directly with strength of UCS and inversely with the CS - UCS interval. In terms of extinction rate, the strongest aversions were the 12.15% aversions at the shortest delays and the weakest aversions were the 2.7% ones. Finally CS - UCS delays longer than 4.5 hr. were not effective in producing aversions at any saline concentration used.

References

Ahlers, R.H. & Best, P.J. Novelty vs. temporal contiguity in learned taste aversions. Psychonomic Science, 1971, 25(1), 34-35.

Balagura, S. & Smith, D.F. External and internal stimulus control of the learned aversion for NaCl in the rat. Proceedings of the 78th. Annual Convention of the American Psychological Association. 1970, 5 (Pt.1), 213-214.

Bauermeister, J.J. & Schaffer, R.W. Relations between preconditioned rate of solution ingestion and rate of post-irradiation intake. Physiology and Behaviour, 1969, 4, 1019-1021.

Best, P.J., Best, M. & Ahlers, R.H. Transfer of discriminated taste aversion to a lever pressing task. Psychonomic Science,1971, 25(5), 281-282.

Best, P.J. & Zuckerman, K. Subcortical mediation of learned taste aversion. Physiology and Behaviour, 1971,7, 317-320.

Boland, F. Saccharin aversions induced by lithium chloride toxicosis in a backward conditioning paradigm. Animal Learning and Behaviour, 1973, 1, 3-4.

Brackbill, R.M. & Brookshire, K.H. Conditioned taste aversions as a function of the number of CS - US pairs. Psychonomic Science, 1971, 22(1), 25-26.

Bradley, R.M. & Mistretta, C.M. Intravascular taste in rats as demonstrated by conditioned aversion to sodium saccharin. Journal of Comparative and Physiological Psychology, 1971, 75(2), 186-189.

Braveman, N.S. & Capretta, P.J. The relative effectiveness of two experimental techniques for the modification of food preferences in rats. Proceedings of the 73rd. Annual Convention of the American Psychological Association, 1965, 1, 129-130.

Brookshire, K. & Brackbill, R.M. Habituation to illness: Effects on acquisition and retention of a conditioned taste aversion. (in prep.).

Capretta, P.J. An experimental modification of food preferences in chickens. Journal of Comparative and Physiological Psychology, 1961, 54, 238-242.

Capretta, P.J. Saccharin consumption under varied conditions of hunger drive. Journal of Comparative and Physiological Psychology, 1962, 55, 656-660.

Domjan, M. & Wilson, N. Specificity of cue to consequence in aversion learning in the rat. Psychonomic Science, 1972, 26(3), 143-145.

Dragonin, W.B. Conditioning and extinction of taste aversions with variations in intensity of the CS and UCS in two strains of rats. Psychonomic Science, 1971, 22(5), 303-305.

Dragonin, W., McCleary, G.E. & McCleary, P. A comparison of two methods of measuring conditioned taste aversions. Behavioural Research Methods and Instrumentation, 1971, 3(6), 309-310.

Edwards, A.L. Experimental Design in Psychological Research. New York: Holt, Rinehart & Winston, 1960.

Essman, W.B. & Alpern, H. Single - trial conditioning: Methodology and results with mice. Psychological Reports, 1964, 14, 731-740.

Farley, J.A., McLaurin, W.A., Scarborough, B.B. & Rawlings, T.D. Pre-irradiation saccharin habituation - a factor in avoidance behaviour. Psychological Reports, 1964, 14, 491-496.

Feinberg, A.M. Saccharin aversion by rats subsequent to treatment with proferrin and x-irradiation. Dissertation Abstracts, 1967, 27, 2869.

Ferguson, G.A. Statistical Analysis in Psychology and Education. Toronto: McGraw-Hill, 1966.

Frumkin, K. Interaction of LiCl aversion and sodium - specific hunger in the adrenalectomized rat. Journal of Comparative and Physiological Psychology, 1971, 75(1), 32-40.

Garcia, J. & Ervin, F.R. Gustatory - visceral and telereceptor-cutaneous conditioning - adaptation in internal and external milieus. Communications in Behavioural Biology, 1968, Part A, 1, 389-415.

Garcia, J., Ervin, F.R. & Koelling, R.A. Learning with prolonged delay of reinforcement. Psychonomic Science, 1966, 5, 121-122.

Garcia, J., Ervin, F.R. & Koelling, R.A. Bait - shyness: A test for toxicity with N = 2. Psychonomic Science, 1967, 7, 245-246.

Garcia, J., Ervin, F.R., Yorke, C.H. & Koelling, R.A. Conditioning with delayed vitamin injections. Science, 1967, 155, 716-718.

Garcia, J., Hankins, W.G., Robinson, J.H. & Vogt, J.L. Bait shyness: Tests of CS - US mediation. Physiology and Behaviour, 1972, 8 (5), 807-810.

Garcia, J. & Kimeldorf, D.J. Temporal relationships within the conditioning of a saccharin aversion through radiation exposure. Journal of Comparative and Physiological Psychology, 1957, 50, 180-183.

Garcia, J. & Kimeldorf, D.J. Some factors which influence radiation conditioned behavior in rats. Radiation Research, 1960, 12, 719-727.

Garcia, J., Kimeldorf, D.J. & Hunt, E.L. Conditioned responses to manipulative procedures resulting from exposure to gamma radiation. Radiation Research, 1956, 5, 79-87.

Garcia, J., Kimeldorf, D.J. & Hunt, E.L. The use of ionizing radiation as a motivating stimulus. Psychological Review, 1961, 68, 383-395.

Garcia, J., Kimeldorf, D.J., Hunt, E.L. & Davies, B.P. Food and water consumption during exposure to gamma radiation. Radiation Research, 1956, 4, 33-41.

Garcia, J., Kimeldorf, D.J. & Koelling, R.A. Conditioned aversion to saccharin resulting from exposure to gamma radiation. Science, 1955, 122, 157-158.

Garcia, J. & Koelling, R.A. Relation of cue to consequence in avoidance learning. Psychonomic Science, 1966, 4, 123-124.

Garcia, J. & Koelling, R.A. A comparison of aversions induced by x-rays, toxins, and drugs in the rat. Radiation Research, 1967, Supplement 7, 439-450.

Garcia, J., Kovner, R. & Green, K.F. Cue properties vs. palatability of flavors in avoidance learning. Psychonomic Science, 1970, 20(5), 313-314.

Garcia, J., McGowan, B.K., Ervin, F.R. & Koelling, R.A. Cues: Their relative effectiveness as a function of the reinforcer. Science, 1968, 160, 794-795.

Gates, J.J. Pre - irradiation food consumption and the saccharin avoidance response. Dissertation Abstracts, 1967, 27, 2892.

Gorry, T. & Ober, S. Stimulus characteristics of learning over long delays in monkeys. Paper presented at the 11th. Annual Meeting of the Psychonomic Society, San Antonio, November, 1970.

Green, K.F. Aversions to grape juice by apomorphine. Psychonomic Science, 1969, 17 (3), 168-169.

Green, K.F. & Churchill, P.A. An effect of flavors on strength of conditioned aversions. Psychonomic Science, 1970, 21 (1), 19-20.

Green, K.F. & Garcia, J. Role of reinforcer in selecting and responding to cues. Proceedings of the 78th. Annual Convention of the American Psychological Association, 1970, 5 (Pt. 1), 251-252.

Green, K.F. & Garcia, J. Recuperation from illness: Flavor enhancement for rats. Science, 1971, 173 749-751.

Green, K.F., McGowan, B.K., Garcia, J. & Ervin, F. Onset of bait - shyness. Proceedings of the 76th. Annual Convention of the American Psychological Association, 1968, 3, 295-296.

Grote, F.W. Jr. & Brown, R.T. Rapid learning of passive avoidance by weanling rats: Conditioned taste aversion. Psychonomic Science, 1971, 25 (3), 163-164.

Hankins, W.G., Garcia, J. & Rusiniak, K.W. Dissociation of odor and taste in bait - shyness. (In prep.).

Hargrave, G.E. & Bolles, R.C. Rat's aversion to flavors following induced illness. Psychonomic Science, 1971, 23 (1B), 91-92.

Harker, G.S. Delay of reward and performance of an instrumental response. Journal of Experimental Psychology, 1956, 51, 303-310.

Harris, L.J., Clay, J., Hargreaves, F. & Ward, A. Appetite and choice of diet: The ability of the vitamin B deficient rat to discriminate between diets containing and lacking the vitamin. Proceedings of the Royal Society of London, Series B, 1933, 113, 161-190.

Hunt, E.L. & Kimeldorf, D.J. The humoral factor in radiation - induced motivation. Radiation Research, 1967, 30, 404-419.

Jacobs, H.L. Studies on sugar preference: I. The preference for glucose solutions and its modification by injection of insulin. Journal of Comparative and Physiological Psychology, 1958, 51, 304-310.

Kalat, J.W. & Rozin, P. "Salience": A factor which can override temporal contiguity in taste - aversion learning. Journal of Comparative and Physiological Psychology, 1970, 71(2), 192-197.

Kalat, J.W. & Rozin, P. Role of interference in taste - aversion learning. Journal of Comparative and Physiological Psychology, 1971, 77(1), 53-58.

Kimeldorf, D.J., Garcia, J. & Rubadeau, D.O. Radiation - induced conditioned avoidance behaviour in rats, mice and cats. Radiation Research, 1960, 12, 710-718.

Kral, P.A. Interpolation of electroconvulsive shock during CS - UCS interval as an impediment to the conditioning of taste aversion. Psychonomic Science, 1970, 19(1), 36-37.

Kral, P.A. Electroconvulsive shock during taste - illness interval: Evidence for induced disassociation. Physiology and Behaviour, 1971, 7, 667-670. (a)

Kral, P.A. ECS between tasting and illness: Effects of current parameters on a taste aversion. Physiology and Behaviour, 1971, 7, 779-782. (b)

Leary, R.W. Food preference changes of monkeys subjected to low-level radiation. Journal of Comparative and Physiological Psychology, 1955, 48, 343-346.

Lovett, D. & Booth, D.A. Four effects of exogenous insulin on food intake. Quarterly Journal of Psychology, 1970, 22, 406-419.

Lovett, D., Goodchild, P. & Booth, D.A. Depression of intake of nutrient by association of its odor with effects of insulin. Psychonomic Science, 1968, 11, 27-28.

Maier, S.F., Zahorik, D. & Albin, R.W. Relative novelty of solid and liquid diet during thiamine deficiency determines development of thiamine - specific hunger. Journal of Comparative and Physiological Psychology, 1971, 74(2), 254-262.

Martin, G. The effect of toxicosis on environment aversion. (In prep.)

Martin, J.C. Saccharin preference and aversion as a function of irradiation and supplier in the albino rat. Psychonomic Science, 1968, 13(5), 251-252.

Martin, J.C. & Ellinwood, E.H. Jr. Aversion to preferred solution after methamphetamine injections. Proceedings of the 79th. Annual Convention of the American Psychological Association, 1971, 6, 769-770.

McGowan, B.K., Garcia, J., Ervin, F.R. & Schwartz, J. Effects of septal lesions on bait - shyness in the rat. Physiology and Behaviour, 1969, 4, 907-909.

McLaurin, W.A. Postirradiation saccharin avoidance in rats as a function of the interval between ingestion and exposure. Journal of Comparative and Physiological Psychology, 1964, 57, 316-317.

McLaurin, W.A., Farley, J.A. & Scarborough, B.B. Inhibitory effect of preirradiation saccharin habituation on conditioned avoidance behaviour. Radiation Research, 1963, 18, 473-478.

McLaurin, W.A., Farley, J.A., Scarborough, B.B. & Rawlings, T.D. Postirradiation saccharin avoidance with non-coincident stimuli. Psychological Reports, 1964, 14, 507-512.

McLaurin, W.A. & Scarborough. B.B. Extension of the inter-stimulus interval in saccharin avoidance conditioning. Radiation Research, 1963, 20, 317-324.

Miller, C.R., Elkins, R.L. & Peacock, L.J. Disruption of a radiation-induced preference shift by hippocampal lesions. Physiology and Behaviour, 1971, 6, 283-285.

Morris, D.D. & Smith, J.C. X-ray conditioned saccharin aversion induced during the immediate postexposure period. Radiation Research, 1964, 21, 513-519.

Mountjoy, P.T. & Roberts, A.E. Radiation - produced avoidance to morphine. Psychonomic Science, 1967, 9, 427-428.

Nachman, M. Learned aversion to the taste of lithium chloride and generalization to other salts. Journal of Comparative and Physiological Psychology, 1963, 56, 343-349.

Nachman, M. Learned taste and temperature aversions due to lithium chloride sickness after temporal delays. Journal of Comparative and Physiological Psychology, 1970, 73, 22-30 (a).

Nachman, M. Limited effects of electroconvulsive shock on memory of taste stimulation. Journal of Comparative and Physiological Psychology, 1970, 73, 31-37 (b).

Nachman, M., Larue, C. & LeMagnen, J. The role of olfactory and orosensory factors in the alcohol preference of inbred strains of mice. Physiology and Behaviour, 1971, 6, 53-59.

Nachman, M., Lester, D. & LeMagnen, J. Alcohol aversion in the rat: Behavioral assessment of noxious drug effects. Science, 1970, 168, 1244-1246.

Ober, S.L. Learned aversions to the taste and colour of ingested substances due to delayed toxicosis in the squirrell monkey. Unpublished masters thesis, Northern Illinois University, 1971.

Pain, J.F. & Booth, D.A. Toxiphobia for odors. Psychonomic Science, 1968, 10(11), 363-364.

Peacock, L.J. & Watson, J.A. Radiation - induced aversion to alcohol. Science, 1964, 143, 1462-1463.

Price, W.R. Aversive behavioral modification with extended inter-stimulus intervals. Dissertation Abstracts International, 1969, 30, 1385-1386.

Revusky, S.H. Aversion to sucrose produced by contingent x-irradiation: Temporal and dosage parameters. Journal of Comparative and Physiological Psychology, 1968, 65, 17-22.

Revusky, S.H. The role of interference in association over a delay. In W.K. Honig & P.H.R. James (Eds.), Animal Memory. New York: Academic Press, 1971.

Revusky, S.H. Basic research relevant to the aversion treatment of alcoholism. (In prep.).

Revusky, S.H. & Bedarf. E.W. Association of illness with prior ingestion of novel foods. Science, 1967, 155, 219-220.

Revusky, S.H. & Garcia, J. Learned associations over long delays. In G.H. Bower & J.T. Spence (Eds.), The psychology of learning and motivation: Advances in research and theory. Vol. 4. New York: Academic Press, 1970.

Revusky, S.H. & Gorry, T. Learned flavor aversions in rats and monkeys with implications for the treatment of alcoholism. (In prep.).

Revusky, S.H., Smith, M.H. Jr. & Chalmers, D.V. Flavor preference: Effects of ingestion - contingent intravenous saline on glucose. Physiology and Behaviour, 1971, 6, 341-343.

Richter, C.P. Increased salt appetite in adrenalectomized rats. American Journal of Physiology, 1936, 115, 155-161.

Richter, C.P., Holt, L.E. & Barelare, B. Vitamin B, craving in rats. Science, 1937, 86, 354-355.

Riege, W.H. Possible olfactory transduction of radiation - induced aversion. Psychonomic Science, 1968, 12(7), 303-304.

Riege, W.H. Disruption of radiation - induced aversion to saccharin by electroconvulsive shock. Physiology and Behaviour, 1969, 4, 157-161.

Rodgers, W. & Rozin, P. Novel food preferences in thiamine - deficient rats. Journal of Comparative and Physiological Psychology, 1966, 61 (1), 1-4.

Roll, D., Schaeffer, R.W. & Smith, J.C. Effects of a conditioned taste aversion on schedule - induced polydipsia. Psychonomic Science, 1969, 16 (1), 39-41.

Roll, D.L. & Smith, J.C. Conditioned taste aversion in anesthetized rats. In M.E.P. Seligman & J. Hager (Eds.), Biological Boundaries of Learning. New York: Appleton - Century - Crofts, 1972.

Rollins, J.B. Jr. Radiation induced aversive conditioning: Dose, dose rate and duration of exposure. Dissertation Abstracts, 1966, 27, 2169.

Rozin, P. Specific aversions as a component of specific hungers. Journal of Comparative and Physiological Psychology. 1967, 64 (2), 237-242.

Rozin, P. Specific aversions and neophobia resulting from vitamin deficiency or poisoning in half - wild and domestic rats. Journal of Comparative and Physiological Psychology. 1968, 66 (1), 82-88.

Rozin, P. Adaptive food sampling patterns in vitamin deficient rats. Journal of Comparative and Physiological Psychology. 1969, 69 (1), 126-132. (a)

Rozin, P. Central or peripheral mediation of learning with long CS - US intervals in the feeding system. Journal of Comparative and Physiological Psychology, 1969, 67, 421-429. (b)

Rozin, P. & Kalat, J.W. Specific hungers and poison avoidance as adaptive specializations of learning. Psychological Review, 1971, 78 (6), 459-486.

Rozin, P. & Ree, P. Long extension of effective CS - US interval by anesthesia between CS and US. Journal of Comparative and Physiological Psychology, 1972, 80 (1), 43-48.

Rozin, P. & Rodgers, W. Novel - diet preference in vitamin deficient rats and rats recovered from vitamin deficiency. Journal of Comparative and Physiological Psychology, 1967, 63(3), 421-428.

Scarborough, B.B. & McLaurin, W.A. Saccharin avoidance conditioning instigated immediately after the exposure period. Radiation Research, 1964, 21, 299-307.

Scarborough, B.3., Whaley, D.L. & Rodgers, J.G. Saccharin avoidance behavior instigated by x-irradiation in backward conditioning paradigms. Psychological Reports, 1964, 14, 475-481.

Schaeffer, R.W., Hunt, E.L. & Kimeldorf, D.J. Application of Premack's theory to a classically conditioned sucrose aversion induced by x-ray exposure. Psychological Record, 1967, 17, 359-367.

Schaeffer, R.W. & Smith, J.C. Lick rates in rats exposed to gamma irradiation. Psychonomic Science, 1966 6(5), 201-202.

Seligman, M.E.P. On the generality of the laws of learning. Psychological Review, 1970, 77(5), 406-418.

Seligman, M.E.P., Mineka, S. & Fillit, H. Conditioned drinking produced by procaine, NaCl and angiotension. Journal of Comparative and Physiological Psychology, 1971, 77(1), 110-121.

Shilling, M. An experimental investigation of the effect of a decrease in the delay of reinforcement upon instrumental response performance. Unpublished master's dissertation, State University of Iowa, 1951. Cited by S.H. Revusky. The role of interference in association over a delay. In W.K. Honig & P.H.R. James (Eds.), Animal Memory. New York: Academic Press, 1971.

Smith, D.F. & Balagura, S. Role of oropharyngeal factors in LiCl aversions. Journal of Comparative and Physiological Psychology, 1969, 69(2), 308-310.

Smith, J.C. & Birkle, R.A. Conditioned aversion to sucrose in rats using x-rays as the unconditioned stimulus. Psychonomic Science, 1966, 5, 271-272.

Smith, J.C. & Morris, D.D. Effects of atropine sulfate on the conditioned aversion to saccharin fluid with x-rays as the unconditioned stimulus. Radiation Research, 1963, 18, 186-190. (a)

Smith, J.C. & Morris, D.D. The use of x-rays as the unconditioned stimulus in five-hundred-day-old rats. Journal of Comparative and Physiological Psychology, 1963, 56 (4), 746-747. (b)

Smith, J.C. & Morris, D.D. The effects of atropine sulfate and physostigmine on the conditioned aversion to saccharin solution with x-rays as the unconditioned stimulus. In T.J. Haley & R.S. Snider (Eds.), Response of the Nervous System to Ionizing Radiation: Second International Symposium. Boston: Little Brown, 1964. (p. 662-672)

Smith, J.C. & Roll, D.L. Trace conditioning with x-rays as the aversive stimulus. Psychonomic Science, 1967, 9, 11-12.

Smith, J.C. & Schaeffer, R.W. Development of water and saccharin preferences after simultaneous exposure to saccharin solution and gamma rays. Journal of Comparative and Physiological Psychology, 1967, 63(3), 434-438.

Smith, J.C., Taylor, H.L., Morris, D.D. & Hendricks, J. Further studies of x-ray conditioned aversion during the postexposure period. Radiation Research, 1965, 24, 423-431.

Smith,R.G. The role of alimentary chemoreceptors in the development of taste aversions. Communications in Behavioral Biology, 1970, 5, 199-204.

St. Omer, V.V. & Kral, P.A. Electroconvulsive shock impedes the learning of taste aversions: Absence of blood - brain - barrier involvement. Psychonomic Science, 1971, 24(5), 251-252.

Stonecypher, J.F. Radiation - induced avoidance conditioning and intracranial self-stimulation. Dissertation Abstracts, 1969, 29, 3117.

Supak, T.D., Marcides, F. & Chorover, S.L. The bait - shyness effect extended to olfactory discrimination. Communications in Behavioral Biology, 1971, 5, 321-324.